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Medically unexplained symptoms and between-group differences in 24-h ambulatory recording of stress physiology

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Abstract

People with medically unexplained symptoms (MUS) often have a comorbid history of stress and negative affect. Although the verbal-cognitive and (peripheral) physiological stress systems have shown a great degree of independence, at the same time it is claimed that chronic stress and negative affect can result in a disregulated physiological stress system, which may lead to MUS. Previous studies could not demonstrate a straightforward between subject relationship between MUS and stress physiology, supporting the view of independence. The aim of the current study was to further explore this relationship using an improved methodology based on ecologically valid 24-h real-life ambulatory recordings. Seventy-four participants (19 male; 55 female) with heterogeneous MUS were compared with 71 healthy controls (26 male; 45 females). Momentary experienced somatic complaints and mood, heart rate, cardiac autonomic activity, respiration and saliva cortisol were monitored using electronic diary and ambulatory registration devices. Participants with MUS reported much more momentary complaints and negative affect as compared to controls. Although MUS seemed to be associated with elevated heart rate and reduced low and very-low frequency heart period variability, these effects disappeared after controlling for differences in sports behaviour. No group differences were found for cardiac autonomic activity, respiration, end-tidal CO_2 and saliva cortisol. Our 24-h real-life ambulatory study did not support the existence of a connection between MUS and disregulated peripheral stress physiology. Future studies may instead focus on central measures to reveal potential abnormalities such as deviant central processing of visceral signals in MUS patients.
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Keywords: Medically unexplained symptoms; Cortisol; Heart rate; PEP; Respiration; RSA

1. Introduction

When somatic symptoms cannot (or not conclusively) be explained by an organic disease, they are considered to be epiphenomena of underlying psychological problems (like anxiety, chronic stress or depression) and are labelled as 'psychosomatic', 'functional somatic' or 'medically unexplained' symptoms (Barsky and Borus, 1999; Costa and McCrae, 1985; Da Costa, 1871; Watson and Pennebaker, 1989; Wessely et al., 1999). Medically unexplained symptoms have a high prevalence and are a burdening problem in primary and secondary health care. These symptoms are more common among women, younger age groups and people from lower

social economic background (Nimnuan et al., 2001a). Several distinct syndromes have been identified such as fibromyalgia, chronic fatigue syndrome, irritable bowel syndrome, multiple chemical sensitivity, noncardiac chest pain and hyperventilation syndrome. However, these syndromes show a considerable overlap and their specificity has been questioned (Barsky and Borus, 1999; Nimnuan et al., 2001b; Wessely et al., 1999).

Medically unexplained symptoms have been associated with stress and negative affect within subjects (changes over time) and between subjects (group differences). Experimental manipulations aimed at inducing somatic complaints (e.g., by inhalation of CO_2-enriched air) in participants high on medically unexplained symptoms showed increased self-reports of distress, state anxiety and negative mood, and experimental manipulations aimed at inducing mental distress showed increased self-reports of somatic complaints (Houtveen et al., 2003; Wientjes and Grossman, 1994). Group differences in medically unexplained symptoms are also closely tied to group differences in current and past reports of anxiety, trauma, neuroticism, negative affect and

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depression (Barsky and Borus, 1999; Houtveen et al., 2003; Pennebaker, 2000; Van Diest et al., 2005; Watson and Pennebaker, 1989; Wessely et al., 1999; Wientjes and Grossman, 1994). The within and between subject relationship between unexplained bodily symptoms and (stress-related) negative affect has been taken as a starting point for the current psychophysiological study.

A negative emotional state may become manifest in the verbal-cognitive system (self-reported measures), the behaviour-motor system (behavioural measures) and in the (peripheral) physiological stress system (stress-physiological measures). The within subject relationship between negative affective states and peripheral stress physiology has been studied extensively. Within-subject changes in self-reported negative emotional state as a result of mental stress clearly coincide with changes in activation of the cardiac sympathetic and parasympathetic divisions of the autonomic nervous system. Mental stress generally leads to increased heart rate, reduced pre-ejection period (PEP) and reduced heart rate variability in the respiratory frequency range (i.e., respiratory sinus arrhythmia; RSA), reflecting increased sympathetic (beta-adrenergic) and decreased parasympathetic (vagal) influences on the heart respectively (Allen and Crowell, 1989; Berntson et al., 1993,1994; Kamphuis and Frowein, 1985; Langewitz and Ruddel, 1989; Sherwood et al., 1986). For most individuals, mental stress also leads to respiratory changes (Boiten et al., 1994; Grossman, 1983) which could result in reduced partial pressure of CO_2 (Han et al., 2000; Ley and Yelich, 1998; Suess et al., 1980). Finally, short-term stress, specifically the stress associated with social evaluation and uncontrollability, gives rise to cortisol elevation (Dickerson et al., 2004).

The between subject association between individual differences in self-reported negative affect (i.e., negative affective traits) and peripheral stress physiology is less clear. A related issue, and of importance for the current study, is whether subjects suffering from medically unexplained symptoms have a disregulated peripheral stress physiology. Frequent and/or intense stressors have been claimed to result in a disregulation of one or more of the physiological stress systems (De Kloet et al., 2005; McEwen, 1998,2003). This is in line with some traditional psychophysiological models of medically unexplained symptoms in which unexplained complaints are suggested to be *reactions* to disregulated (i.e., exaggerated) peripheral manifestations of stress and anxiety (Sharpe and Bass, 1992). This concerns, for example, the role of muscle tension in low back pain, respiration and CO_2 in the hyperventilation syndrome, and disruptions of the autonomic balance or the stress hormone cortisol in chronic fatigue syndrome. It has, however, frequently been observed that the verbal-cognitive and the physiological stress systems show a great degree of independence (Lang, 1994; Wilhelm and Roth, 2001). The discordance between self-reported and stress-physiological measures may be especially true when comparing subjects. Previous between subject (group-comparing) studies on the relationship between medically unexplained symptoms and stress-physiological measures have shown mixed results. Although in some of these studies autonomic, respiratory or

HPA-axes irregularities have been reported in relation to medically unexplained syndromes and anxiety disorders (Bystritsky et al., 2000; Coryell et al., 2001; Heim et al., 2000; Hoehn-Saric et al., 1991; Thayer and Brosschot, 2005; Wilhelm et al., 2001), many null-findings have also been reported (Hornsveld et al., 1996; Houtveen et al., 2003; Kirschbaum et al., 1992; Mommersteeg et al., 2006a,b; Troosters et al., 1999; Wientjes and Grossman, 1994). Thus, although efforts to demonstrate a between subject correspondence between self-reported medically unexplained symptoms, stress, anxiety on the one hand and peripheral stress physiology on the other have not yet been very successful in the past, this issue remains of importance for validation or falsification of the traditional psychophysiological models that claim a role for disregulated stress physiology to explain medically unexplained symptoms.

The aim of the current study was to further explore the between subject relationship between medically unexplained symptoms and peripheral stress physiology using an improved methodology. Physiological measures are traditionally taken in a laboratory situation under artificial baseline conditions or in response to artificial stressors. However, this way of testing has a low ecological validity. For example, generalisation of individual differences in cardiovascular stress reactivity from laboratory situations to real-life situations has been shown to be moderate at best (Gerin et al., 1994; Kamarck et al., 2003; van Doornen et al., 1994). Moreover, circadian variation has been demonstrated in baseline and reactivity values of cardiac autonomic levels (van Eekelen et al., 2004a,b), respiration (Mortola, 2004) and HPA axis activity (Buijs, 1999; Van Eekelen et al., 2003), and physiological group differences may be specifically manifest in specific windows of the diurnal cycle. For these reasons (ecological validity of the test situation and the possibility of circadian variation of group differences), between subject differences in physiological stress profiles preferably should be assessed in a real-life situation by using ambulatory measurement devices and last a full circadian time-frame. As an example of the utility of this approach, Vrijkotte et al. (2000) found that men reporting high work stress (classified with Siegrist's model as an imbalance between high effort and low reward at the workplace) had increased heart rates, lower heart period variability and increased sleep-leisure-work differences as compared to men low in stress (Vrijkotte et al., 2004). These results show how ambulatory recordings can reveal stress-physiological differences related to between subject differences in self-reported mental stress.

In the current 24-h ambulatory study, self-reported measures and stress-physiological measures were assessed simultaneously. The aim of the current study was to relate group differences in medically unexplained symptoms to group differences in (the set-points of) stress physiology. Momentary experienced somatic complaints and mood were assessed with electronic diaries to demonstrate the presence of symptoms and negative emotional states during the measurement day. The use of a more reliable assessment of complaints by way of a diary excludes the possibility that an absence of the expected relationship is due to the role of retrospective bias when using

questionnaires. As stress-physiological indices we opted for the activity of the (cardiac) autonomic nervous system, the respiratory system, and the hypothalamic–pituitary–adrenal (HPA) axis. All measures were assessed on the basis of real-life 24-h ambulatory recordings. Group differences on one or more of these physiological systems should become observable in either base levels or day–night reactivity patterns.

2. Method

2.1. Participants

Participants with medically unexplained symptoms ($n = 74$; 19 male, 55 female) were recruited by linking national websites on medically unexplained syndromes (e.g., hyperventilation syndrome, chronic fatigue syndrome, clinical burnout syndrome) to research information provided through the university website (http://www.fss.uu.nl\gp\stressprofielen). People who responded (by electronic mail) received additional information and the inclusion questionnaires (see below). Participants with complaints were included based on high scores on the somatisation subscale of the SCL-90-R in combination with the absence of a medically diagnosed physical disease (see below). The control participants ($n = 71$; 26 male, 45 female) were recruited through the internet, advertisements in local papers, or were nearby volunteers. See Table 1 for further participant information. The study was presented as an investigation of stress profiles.

Inclusion criteria for the group high on symptoms were: age 25–50, scoring ≥27 on the somatic subscale of the Symptom Check List (SCL-90-R), ≤33 on the anxiety subscale and ≤55 on the depression subscale, and a self-report of having consulted a physician for their somatic complaints. With these criteria, we tried to include candidates with somatic complaints without organic cause, but without a primary anxiety or depressive disorder. Inclusion criteria for the controls were: age 25–50, ≤18 on somatic subscale, ≤14 on anxiety and ≤23 on depression. Exclusion criteria for all participants were the use of medication known to affect our physiological measures (e.g., corticosteroids, anti-depressives, beta-blockers), a medically diagnosed physical disease, a body mass index (BMI) > 30, and excessive use of alcohol or drugs. Participants high on symptoms received a small present afterwards; control participants low on symptoms received a small monetary reward (10€). All participants received an

annotated summary of their recordings. The Ethics Committee of the University Medical Centrum Utrecht (UMCU) approved the study protocol and all subjects gave written consent before entering the study.

2.2. Inclusion questionnaires

A Dutch translation of the Symptom Check List (SCL-90-R) (Arrindell and Ettema, 1981) was used for the selection of participants. Distress ratings of a list of items experienced in the previous week could be rated on a five-point Likert scale, ranging from 1 '*not at all*' to 5 '*extremely*'. The subscales used were somatisation ($n = 12$ items; see Appendix A) anxiety ($n = 10$ items) and depression ($n = 16$ items).

A second questionnaire was made to evaluate demographic information (age, length, weight, work status, engagement in sports, etc.) and self-reported health status. Participants were asked to report their recent experienced illness, medical history and previous and current medication use. For 18 medical problems (e.g., pain, inflammations, heart problems, asthma) questions had to be completed regarding experiences in the last 6 months: whether a physician was ever seen for this complaint, a medical diagnosis was ever received, or a treatment (e.g., medication) for this complaint was prescribed.

2.3. Ambulatory measurement devices

The ambulatory electrocardiogram (ECG) and impedance cardiogram (ICG) were measured from a six Ag/AgCl electrode configuration using the VU-AMS (version 4.3, TD-FPP, Vrije Universiteit, Amsterdam, The Netherlands; http://www.psy.vu.nl/vu-ams). Electrode resistance was kept low by cleaning the skin with alcohol and rubbing. Vertical acceleration of the torso – integrated over 30-s periods – was additionally monitored and stored throughout the 24-h recording time. This was used as a proxy for gross body movement (motility). For reasons of memory limitations, continuous registration was set to 5 min per 15 min period, resulting in four data segments per hour. Details on the recording methodology, reliability and validity of the VU-AMS can be found elsewhere (De Geus and Van Doornen, 1996; De Geus et al., 1995; Houtveen et al., 2006; Riese et al., 2003; Willemsen et al., 1996).

Palm[TM] M130 (http://www.PalmOne.com) Personal Digital Assistants (PDA) were used for (electronic diary) experience sampling of symptoms and mood during the measurement day. Special software was developed for the generation of alarms (a 10 s auditory signal) during the day, and for the assessments of somatic complaints and mood. A fixed sampling protocol with an interval of 1.5 h was used that continued from awakening till bedtime. This sampling scheme leads to approximately 11 alarms per day (based on a 16 h awakening period). Diary prompting was only disabled during sleep, initiated by a button on the PDA. Next, the PDA could be used as a morning alarm and prompting continued after awakening. All unused buttons were blocked. Alarms without response were repeated (maximum three times with 3 min time interval). The alarm software generated a log-file with alarm and response times. This file was used for determination of compliance. The questionnaire was launched by a start button that was visible for 12 min after prompting. All questions were forced-choice, and they were displayed as sequential screens on the PDA. Participants were not allowed to leaf through the present or previous diaries. The self-reported dimensions measured on the PDA were: activity and posture, somatic complaints (measured by the 12 somatic complaint items used for selection based on de somatisation subscale of the SCL-90-R; see appendix) and mood (i.e., depression, vitality, anger, fatigue and tension; each measured by three items adapted from the shortened version of the POMS (Shacham, 1983); see appendix). Each item could be rated on a seven-point scale ranging from 1 '*not at all*' to 7 '*very much*'.

Together with the use of the PDA, the TG-951T CO_2 quantitative sensor Kit (Nihon Kohden Corporation, Tokyo, Japan) was used to measure the partial pressure of CO_2 at the end of a normal expiration (PetCO$_2$). PetCO$_2$ was measured during the day using a mainstream adapter (based on 4 respiratory cycles assessed once every 1.5 h automatically initiated just after completing the diary questions) and during sleep using an air pump device in combination with a sidestream (nostrils) adapter (based on 10 respiratory cycles assessed once every 15 min; we anticipated more loss of data as a result of mouth breathing during sleep). Digitized PetCO$_2$ values (in mmHg) from the capnometer were sent to the PDA computer for storage through an RS-232 interface cable.

Table 1
Participant information

	LSS ($n = 71$)	HSS ($n = 74$)	Significance
Male (%)	36	26	ns
Smoking (%)	25	39	ns
Engaged in sports (%)	73	54	<.05
Employed (%)	90	64	<.001
Receive benefits from social security administration (%)[a]	0	19	<.001
Unemployed[b]	10	17	ns
On sick leave (%)[c]	0	28	<.001

	M	S.D.	M	S.D.	Significance
Age (years)	34.61	7.86	36.18	8.04	ns
BMI (kg/m^2)	22.91	2.46	23.57	3.80	ns
SCL90R Som	14.34	1.68	33.27	7.28	< .001
SCL90R Anx	11.18	1.37	23.22	8.09	< .001
SCL90R Dep	18.25	2.21	36.38	9.59	< .001

Notes: LSS, low on somatic symptoms; HSS, high on somatic symptoms; ns, not significant.

[a] Unemployed because of chronic (mental) disability as a result of medical unexplained symptoms.

[b] Unemployed for other reasons (not as a result of having complaints).

[c] Employed but on sick leave whilst being assessed.

Finally, the PDA prompted times for the collection of saliva. Saliva was collected by salivettes, plastic tubes with cotton roles (Sarstedt, Etten-Leur, the Netherlands). Saliva was collected at 0, 15, 30 min after awakening for the cortisol awakening response and at noon, 6 pm and 11 pm (or sleeping time) for the day-curve. Using the PDA for prompting of saliva collection times allowed us to assess cortisol-influencing parameters as food intake, smoking, teeth brushing and medication. The cortisol alarm was repeated after recent food intake, smoking, or teeth brushing. The samples were kept in the refrigerator after collection and (after being recollected 24-h later) stored at −20 °C. Samples were analysed in a lab in Dusseldorf (Germany), by a time-resolved immunoassay with fluorescence detection as described elsewhere (Dressendorfer et al., 1992).

2.4. Procedure

Participants who met the inclusion criteria and who agreed to participate were contacted by telephone to receive more information about the study and to make an appointment for the 24-h ambulatory recordings. They were visited at home where they completed additional questionnaires. Next, they were supplied with the VU-AMS, the PDA, the capnometer, and the salivettes. They received detailed verbal and written instructions how to use all equipment. Participants practised how to complete the questions on the PDA. Next, they were instructed on the CO_2 assessment. Specific instructions were given how to breath as normal as possible during mainstream CO_2 assessments, which was also practiced under supervision. They got further instructions on how to regularly check the 'all clear' signal of the VU-AMS device (a small blinking light on the side of the device) and how to respond to measurement alarms (e.g., a loose electrode contact) and electronic diary promptings. Twenty-four hour telephone assistance was available. Participants followed their normal day (e.g., working) routines. The following day, the researcher came back to collect the devices.

2.5. Physiological data analyses

The recorded heart period time series (inter-beat intervals, IBI) together with the motility signal were displayed as a cardiotachogram for visual inspection by two independent raters. The (5-min) segments containing too many artifacts (i.e., >5) were reduced in length (sub-segments low in artefacts should be at least 2.5 min) or rejected (no sub-segments could be selected). Because individual differences in motility could be responsible for group differences in the physiological measures, only segments (or sub-segments 2.5–5 min) low on motility were selected. 'Low on motility' was based on the mean participant-specific motility value obtained during sleep. In 89% of the cases, a (sub)segment low in motility could be found. Note that low motility (sub)segments were only found during sleep (by definition) and (whilst awake) during relaxation in sitting or supine position. These (sub)segments were used for segment-specific analysis of the IBI and thoracic impedance (ICG) signals.

An artifact pre-processing was performed on the selected IBI data. Artifacts were detected automatically when greater than a user-predefined percentage of: (1) the standard deviation or (2) the mean value in deviation from the moving mean of a particular segment, and accepted or overruled by visual inspection. IBI artifacts were found and corrected for .07% (S.D. = .06) of all IBI values. Since artifacts cannot simply be deleted (i.e., the continuity of time would be lost) spuriously short IBIs were summed and missing beats were 'created' by splitting spuriously long IBIs. For each segment, the IBI mean values were computed from these corrected data.

Next, uniformly spaced samples were created by interpolation of the corrected IBI data using a Wavelet interpolation algorithm. Discrete Wavelet Transformation (DWT) was performed using a cardinal cubic spline function as base. This method results in identical power values for stationary relatively short data segments as compared to Fourier transformation (Houtveen and Molenaar, 2001), but it is superior for non-stationary data segments. Since the DWT (like Fourier) suffers from aliasing effects at both ends, the first and last 2.5 s of the time series were excluded from the derivation of the variances. The High Frequency (HF) power (the variance in the .125–.5 Hz window) was computed as main measure of cardiac parasympathetic (vagal) control (Berntson et al., 1997). The Low Frequency (LF) power (.0625–.125 Hz) and the Very-Low Frequency (VLF) power (.0078125–.0625 Hz) were computed as explorative measures. The LF power indicates a complex sympathetic and

parasympathetic cardiac regulation possibly related to the blood pressure rhythm. Potential stimuli and conditions that may contribute to VLF rhythms vary from thermoregulatory to the rennin–angiotensin system (see Berntson et al., 1997 for a review).

Changes in the thoracic impedance (dZ) were used to asses respiration. Respiratory-related changes in dZ were obtained by band-pass filtering (.125–.5 Hz) of the ICG signal using a DWT filter with a cubic spline function as base. Next, the respiratory power values were computed as the variances of the filtered time series for each (selected) data segment. Changes in the respiratory power values were used as a (raw) estimation of changes in respiratory depth as described elsewhere (Houtveen et al., 2006). Respiratory frequencies were obtained from the band-pass filtered thoracic impedance (dZ) signal by counting the number of up-going zero crossings and dividing this value by the time of a segment as described elsewhere (De Geus et al., 1995; Houtveen et al., 2006).

Pre-ejection period was manually scored using the VU-AMS interactive software which graphically displays the large-scale ensemble averages (i.e., averaged per segment). Pre-ejection period reflects the time interval between the onset of the electromechanical systole (Q-wave onset) in the ECG and the onset of left ventricular ejection at the opening of the aortic valves (B-point) in the ICG. The B-points were manually determined for each ensemble averaged (selected) segment, and the pre-ejection period values were determined by summing a fixed Q-to-R interval of 48 ms to the R-B interval time. Pre-ejection period was computed as measure of cardiac sympathetic control (inotropic control over cardiac contractility) (Sherwood et al., 1990).

$PetCO_2$ values were assessed with 1.5 h intervals during daytime (based on 4 respiratory cycles) and 15 min intervals during sleep (based on 10 respiratory cycles). The largest value of the respiratory cycles (4 during the day; 10 during the night) was taken as the most reliable value (i.e., based on the idea that an end-tidal value could not exceed the true arterial partial pressure of CO_2). Visual inspection was performed on the sleep data to reject incidental low values due to mouth breathing. Suspicious values were evaluated in combination with respiratory depth and frequency.

2.6. Statistical analyses

Electronic diary data were mean-aggregated over four 4-h time periods (8–12 h, 12–16 h, 16–20 h and 20–24 h); physiological data were mean-aggregated over six 4-h time periods (including 0–4 h and 4–8 h). The electronic diary data, IBI, heart period variability powers, respiratory power, pre-ejection period and cortisol values were ^{10}log transformed to obtain normal distributions. Repeated measures Group (2) by Gender (2) by Time-Period (4 or 6) analysis of variance tests (using the MANOVA approach, SPSS 14) were performed for all measures. P-values < .05 were considered significant.

3. Results

See Table 2 for the mean number of aggregated responses per time period (across subjects) and the corresponding across subjects means (and S.D.) of the aggregated physiological measures assessed.

3.1. Electronic diary

The mean number of diary (and day-time CO_2) responses during a day was 11.06 (participants high on symptoms: $M = 11.19$, range 10–13; low on symptoms: $M = 10.93$, range 8–13), no significant group differences were found, $T(143) = −1.47$, $p = .14$.

See Fig. 1 for group differences in the reported momentary experienced somatic complaints and mood. Participants high on symptoms reported significantly more somatic complaints, $F(1,140) = 172.70$, $p < .001$, more depression, $F(1,140) = 30.70$, $p < .001$, less vitality, $F(1,140) = 70.15$, $p < .001$, more anger, $F(1,140) = 26.95$, $p < .001$, more fatigue, $F(1,140) =$

Table 2
Mean number of responses that were aggregated (n_{agg}) and means (S.D.) of the physiological measures

	0–4 h		4–8 h		8–12 h		12–16 h		16–20 h		20–24 h	
	LSS	HSS	LSS	HSS	LSS	HSS	LSS	HSS	LSS	HSS	LSS	HSS
n_{agg} diary					2.4	2.5	2.9	3.1	3.0	3.0	2.6	2.6
n_{agg} vu-ams	15.6	15.8	15.1	15.5	13.1	13.2	13.7	12.8	12.9	14.0	14.0	14.5
IBI	972 (144)	916 (160)	995 (133)	927 (133)	834 (126)	774 (104)	809 (112)	750 (105)	821 (118)	762 (102)	843 (127)	806 (130)
HF-power	2.63 (.45)	2.61 (.45)	2.71 (.45)	2.67 (.40)	2.64 (.38)	2.55 (.35)	2.60 (.37)	2.52 (.34)	2.58 (.40)	2.52 (.37)	2.58 (.45)	2.54 (.36)
LF-power	2.78 (.37)	2.66 (.43)	2.87 (.34)	2.78 (.38)	2.89 (.27)	2.76 (.30)	2.84 (.28)	2.75 (.30)	2.84 (.31)	2.71 (.31)	2.83 (.33)	2.68 (.32)
VLF-power	3.23 (.29)	3.12 (.31)	3.41 (.27)	3.29 (.31)	3.22 (.23)	3.08 (.26)	3.14 (.25)	3.016 (.27)	3.15 (.28)	2.98 (.28)	3.13 (.29)	2.99 (.26)
Resp-power	3.45 (.38)	3.54 (.31)	3.54 (.29)	3.56 (.29)	3.80 (.25)	3.85 (.23	3.84 (.23)	3.91 (.22)	3.89 (.22)	3.89 (.22)	3.85 (.28)	3.84 (.26)
Resp-freq	.27 (.035)	.27 (.040)	.27 (.03)	.27 (.04)	.29 (.02)	.29 (.02)	.29 (.02)	.30 (.03)	.30 (.018)	.30 (.03)	.30 (.03)	.30 (.03)
PEP	88.9 (4.3)	87.3 (5.1)	89.1 (4.5)	87.5 (5.0)	87.8 (4.9)	86.0 (3.8)	87.0 (5.4)	85.4 (4.1)	87.4 (5.2)	85.4 (5.0)	86.5 (4.5)	85.2 (4.3)
n_{agg} PetCO$_2$	13.4	12.4	11.4	9.9	3.2	3.2	3.0	3.1	3.0	3.0	3.7	4.5
PetCO$_2$	37.4 (3.7)	37.0 (3.4)	37.4 (3.6)	37.1 (3.5)	35.9 (4.5)	35.7 (4.3)	35.5 (4.4)	35.5 (4.4)	35.3 (4.0)	35.9 (4.3)	37.2 (4.1)	36.8 (3.7)

	Awake		+15 min		+30 min		12:00		18:00		Bed time	
	LSS	HSS	LSS	HSS	LSS	HSS	LSS	HSS	LSS	HSS	LSS	HSS
Cortisol	13.9 (4.8)	13.9 (5.7)	19.6 (7.0)	20.5 (7.8)	20.4 (8.5)	21.5 (8.2)	8.5 (4.2)	8.9 (5.0)	4.1 (2.6)	4.3 (2.4)	2.0 (2.9)	1.8 (2.0)

Notes: LSS, low on somatic symptoms; HSS, high on somatic symptoms; IBI, inter beat interval (ms); heart period (HF, LF, VLF) and respiratory power (Resp-power) values were [10]log transformed; Resp-freq, respiratory frequency (Hz); PEP, pre-ejection period (ms), PetCO$_2$, end-tidal partial pressure of CO_2 (mmHg); cortisol, saliva cortisol (nmol/l).
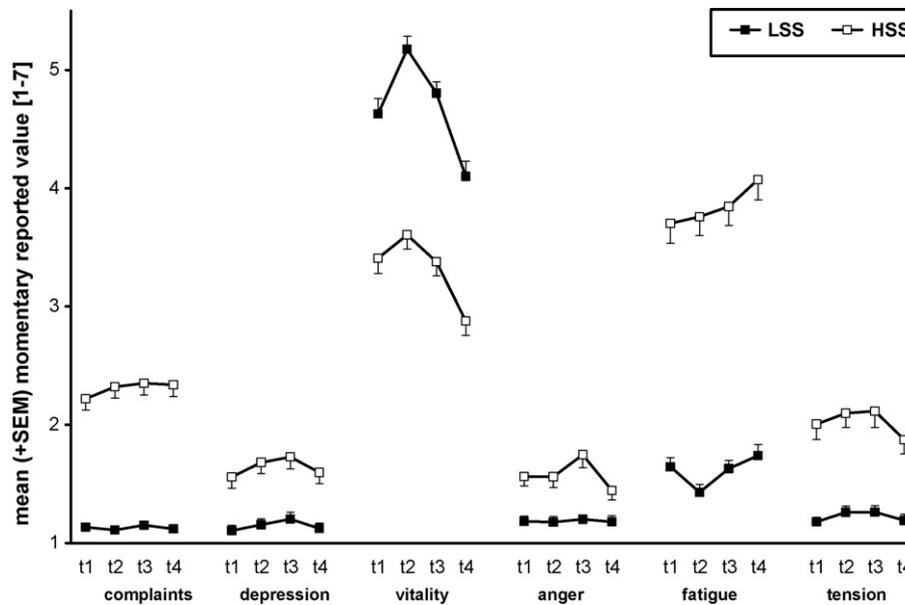
Fig. 1. Group differences for momentary experienced somatic complaints and mood. *Notes*: LSS, low on somatic symptoms; HSS, high on somatic symptoms; $t1 = 8–12$ h; $t2 = 12–16$ h; $t3 = 16–20$ h; $t4 = 20–24$ h.

172.18, $p < .001$ and more tension, $F(1,140) = 47.15$, $p < .001$. No significant effects of Gender were found. Significant effects of Time-Period were found for vitality, Wilks' $\lambda = .64$, $F(3,138) = 26.48$, $p < .001$ (post-hoc tests yielded less vitality in the evening hours), fatigue, Wilks' $\lambda = .92$, $F(3,138) = 4.07$, $p < .01$ (post-hoc tests yielded more fatigue in the evening hours) and tension, Wilks' $\lambda = .93$, $F(3,138) = 3.53$, $p < .05$ (post-hoc tests yielded more tension in the afternoon hours, 12–20 h). Only one significant interaction effect was found: participants high on symptoms reported less somatic symptoms during the morning hours, Wilks' $\lambda = .94$, $F(3,138) = 3.07$, $p < .05$.

Fig. 2. Group differences for 24-h mean IBI values for (a) all participants (left) and (b) with exclusion of men engaged in sports (right). *Notes*: LSS, low on somatic symptoms; HSS, high on somatic symptoms; ns, not significant.

3.2. Cardiac measures

Participants high on symptoms had significantly shorter IBI values (i.e., a higher heart rate), $F(1,141) = 18.56$, $p < .001$, lower LF heart period power values, $F(1,141) = 4.03$, $p < .05$, and lower VLF heart period power values, $F(1,141) = 9.14$, $p < .01$ (see Figs. 2a and 3a). One significant interaction effect between Group and Gender was found, $F(1,141) = 6.41$, $p < .05$: the IBI group effect found was more pronounced for men as compared to women. Main effects of Group were not found for HF power and pre-ejection period.

Significant main effects of Gender were found for IBI, $F(1,141) = 18.56$, $p < .001$, LF power, $F(1,141) = 14.74$, $p < .001$, VLF power, $F(1,141) = 18.10$, $p < .001$, and pre-ejection period, $F(1,141) = 9.02$, $p < .01$. Women had shorter IBI values, lower LF and VLF heart period power values and shorter pre-ejection period values as compared to men. Significant Time-Period effects were found for all cardiac measures (all p's $< .01$). IBI, VLF, LF and HF heart period power values, and pre-ejection periods were higher during sleep (i.e., at night time).

Additional tests were performed to compensate for possible confounders affecting the observed cardiac effects. First, smoking effects were found for IBI and the heart period variability power values (p's $< .001$). Smokers had shorter IBI values and lower heart period variability power values. Smoking was not related to group and could, therefore, be used in an analysis of covariance to improve the power of the tests (see Miller and Chapman, 2001). This, however, did not alter significances of the statistical tests for group differences in IBI and heart period power values. Second, the gender composition of the groups was not completely equal and the groups differed in the percentage of persons who were regularly engaged in sports (see Table 1). These two group differences were related since men were over-represented in the group low
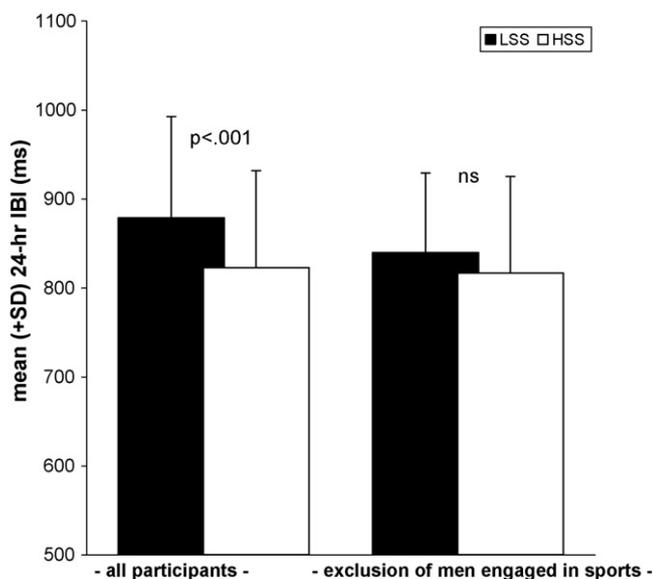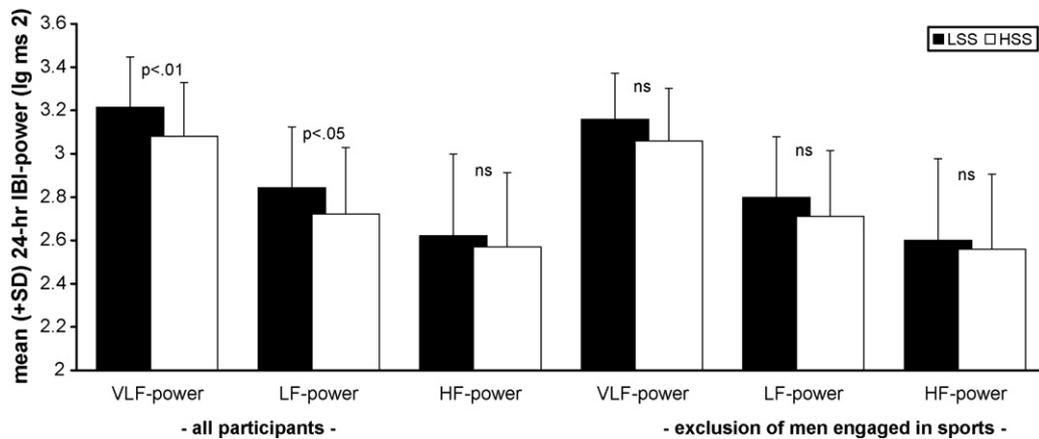
Fig. 3. Group differences for 24-h mean VLF, LF and HF-power values for (a) all participants (left) and (b) with exclusion of men engaged in sports (right). *Notes*: LSS, low on somatic symptoms; HSS, high on somatic symptoms; ns, not significant.

on symptoms whereas more men low on symptoms reported to be engaged in sports ($n = 18$) as compared to men in the 'high-symptoms/engaged in sports' subgroup ($n = 8$). Men in the 'low-symptoms/engaged in sports' subgroup had much larger IBI values (24-h mean IBI values = 996) as compared to the other participants (overall mean excluding men low on symptoms and engaged in sports = 835, range 791–889). Increased VLF and LF power values were found for this subgroup as well. Engaging in sports on a regular basis could, since it was related to group, not be used as a covariate to 'control' for this undesired group difference (see Miller and Chapman, 2001). Therefore, we performed additional repeated measures tests excluding all men who engaged in sports ($n = 26$). Notify that for the remaining 119 participants, men and women were equally distributed across the groups (i.e., 15% versus 17% men). Mean 24-h IBI and heart period variability power values are displayed for this reduced data set in Figs. 2b and 3b. Repeated measures tests did not show significant cardiac effects of Group anymore (i.e., all p's > .05). Random exclusion of six women of the control group who engaged in sports (i.e., to remove the smaller and non-significant group differences on sports behaviour for the female participants) did not show significant cardiac effects of Group either.

3.3. Respiration and PetCO$_2$

No significant main effects of Group were found for respiratory power, respiratory frequency and PetCO$_2$. Significant effects of Gender were, however, found for all respiratory measures. Women had lower respiratory power values, $F(1,140) = 9.12$, $p < .001$, higher respiratory frequency values, $F(1,140) = 5.32$, $p < .05$, and lower PetCO$_2$ values, $F(1,134) = 33.81$, $p < .001$, as compared to men. Significant effects of Time-Period were found for all respiratory measures (all p's < .001); respiratory power and frequency values were lower at night time whilst PetCO$_2$ was higher. Additional repeated measures tests excluding all men engaging in sports yielded similar results for respiratory power and PetCO$_2$.

3.4. Cortisol

No significant main effects of Group or Gender were found for cortisol. The obvious effects of Time-Period were found, Wilks' $\lambda = .06$, $F(5,132) = 393.85$, $p < .001$: higher cortisol values for +15 min and +30 min as compared to awakening time (i.e. the cortisol awakening response) and a lowering of cortisol as time passes by during the day. Additional repeated measures tests excluding all men engaging in sports yielded similar results.

4. Discussion

Groups of participants were selected based on high versus low scores on recently experienced *retrospectively* reported medically unexplained somatic symptoms. During the 24-h measurement period, group differences in *momentary* experienced somatic complaints and negative mood were, as expected, large as well. These group differences in self-reported symptoms and negative emotional states were not reflected in 24-h ambulatory recordings of HF heart period variability power as a measure of parasympathetic (vagal) control of heart rate and pre-ejection period as measure of sympathetic (inotropic) control over cardiac contractility. Thus, a deviant baseline or day-night reactivity pattern in cardiac autonomic activity could not be demonstrated for the group high on medically unexplained symptoms.

Reduced 24-h baseline values of IBI, LF and VLF powers were found for participants high on symptoms. The heart period effect was found in the absence of sympathetic and vagal differences. This suggests a group difference in intrinsic heart period (see Berntson et al., 1993), for example as a result of group differences in physical fitness or in gender composition of the groups. A further exploration indeed showed that these group differences were not found to be significant anymore after the exclusion of men who reported to be engaged in sports on a regular basis (see Figs. 2 and 3). In these additional analyses, the groups did not differ in gender composition anymore, thereby also ruling out a possible effect of gender on the results.

It is recommended to take individual differences in physical fitness into account when demonstrating a between subject relationship with stress physiology (see also Cook et al., 2006). Relatively low heart rate and high heart rate variability values have been related to aerobic fitness in the literature. Habitual aerobic exercise has been demonstrated to play a role in the maintenance of augmented heart rate variability (measured during paced breathing at .1 Hz; the LF frequency band) in active men when compared with age and weight matched sedentary controls (Edmond and Meersman, 1993). Other 24-h ambulatory recording studies have shown that reduced LF and VLF heart rate variability, observed in a group of older men and women who were initially sedentary, increased after a 6-month physical training program (De Meersman, 1993; Schuit et al., 1999). Studies on the effect of fitness that focused on the HF band did not find any effect (De Geus et al., 1996). Additionally, it has been stated that the decline in cardiac vagal modulation often attributed to increasing age may, instead, be the result of a decline in fitness (Goldsmith et al., 1997). Because regular exercise has cross-sectionally been associated with lower neuroticism, anxiety and depression (De Moor et al., 2006), subjects suffering from medically unexplained symptoms – with high scores on anxiety, depression and neuroticism (Feldman et al., 1999) – are most likely less engaged in sports on a regular basis as well. This may result in higher heart rates and lower heart rate variability values. It remains unclear why specifically the lower frequency bands (LF and VLF) were affected by physical fitness. Nonetheless, further explanation of the frequency-specificity of this effect seems irrelevant in the present framework.

Smoking causes an acute and a long-term effect on cardiac autonomic regulation (Hayano et al., 1990). This was supported by the findings of the current study where heart period and heart period variability powers were lower for the smokers. Because adding smoking as a covariate did not alter significances, it can be concluded that smoking is an independent factor, not related to medically unexplained symptoms, that does influence cardiac autonomic activity.

Respiratory depth, respiratory frequency, $PetCO_2$ and cortisol levels were recorded as other potential candidates to demonstrate between subject differences related to stress and stress-related disorders. Respiratory behaviour was also measured because respiration can potentially influence HF heart period variability independent of cardiac parasympathetic (vagal) control (Berntson et al., 1997; Houtveen et al., 2002). Group differences were, however, not found for the 24-h ambulatory recordings of respiratory behaviour. It is therefore unlikely that group differences in respiratory behaviour could have masked differences in heart period variability. Group differences were not found for saliva cortisol either (awakening response and day-curve). Demonstrating between-group differences in cortisol level related to stress has shown to be complicated as well. Even for a syndrome as clinical burnout, which evidently is the product of long-standing exposure to work stress, no effects on cortisol parameters could be demonstrated (Mommersteeg et al., 2006a).

Ambulatory stress-physiological measurements can be used for assessment of situation-specific physiological reactivity (cardiovascular, breathing patterns and HPA-axis responses) in real-life situations, like the physiological anxiety response to a phobic situation or the physiological responses to (work) stress. Within subject and between subject comparisons can be made on situation-specific physiological reactivity in real-life situations (see also Wilhelm et al., 2006). The ambulatory methodology also allows between subject comparison of physiological base levels or day–night reactivity patterns. The results of the current study, however, do not demonstrate straightforward between subject differences related to medically unexplained symptoms and negative affect in the physiological stress parameters assessed. Thus, ambulatory stress-physiological measurements on subjects suffering from medically unexplained symptoms may have their main application in the assessment of within subject, situation-specific physiological reactivity in real-life situations.

The participants in the current study were not categorized into specific medically unexplained syndromes. We have not performed such a categorisation because there are no objective diagnostic criteria for most of the medically unexplained syndromes known; they have a large overlap in symptoms and non-symptom characteristics (e.g., co-morbid psychiatric problems like anxiety and depression) (Wessely et al., 1999). Participants high on a variety of unexplained somatic complaints were therefore included based on the somatisation subscale of the SCL-90-R in combination with the absence of a medically diagnosed physical disease. Health status of the participants was based on self-reports only. However, patients suffering from numerous medically unexplained symptoms are known for frequent medical consultations (known as 'doctor shopping') (Barsky and Borus, 1999) and they were expected to embrace and report any regular medical explanation for their complaints immediately. Nonetheless, the fact that participants were not diagnosed by an independent physician can be considered a potential limitation of the current study.

The estimation of respiratory depth and frequency was based on changes in the thoracic impedance signal (without controlling for the effects of talking), which may be a limitation of the current study. Although the thoracic impedance methodology used was validated as an instrument to assess individual differences in respiratory frequency, tidal volume assessments are less accurate (Houtveen et al., 2006). Our cardiac sympathetic and vagal, $PetCO_2$, electronic diary and cortisol in saliva technology was highly advanced and more than adequate to detect potential individual differences in these measures. Nonetheless, group differences may exist in other physiological parameters such as immune function or in the metabolic system. Additionally, because physiological activation patterns are affected by individual response specificity (Wilhelm and Roth, 2001), specific subgroups with medically unexplained symptoms might exist that differ on subgroup-specific patterns of physiological disregulation. It is concluded that, for the physiological measures and analysis methods used (i.e., keeping the limitations into account), between subjects concordance between unexplained symptoms (related to stress and anxiety) and peripheral stress physiology could not be demonstrated.

Experimental studies have indicated that medically unexplained symptoms may be the result of a sensitisation to normal interoceptive (stress-physiological) signals, possibly caused by stress-related deviations in the immune-to-brain communication system (Dantzer, 2005; Wieseler-Frank et al., 2005) or in central mechanisms related to the perception of interoceptive signals (Craig, 2002,2003; Hoehn-Saric et al., 2004; Verne et al., 2004). These approaches, considering our results as presented, hold more promise to unravel potential (central) physiological disregulations related to medically unexplained symptoms than a focus on peripheral stress-physiological indices.

Acknowledgements

Appendix A

The 12 items of the somatisation subscale of the SCL-90-R used for the current study are: (1) headache, (2) faintness or dizziness, (3) pain in heart or chest, (4) pain in lower back, (5) nausea or upset stomach, (6) soreness of muscles, (7) trouble getting your breath, (8) hot or cold spells, (9) numbness or tingling in part of the body, (10) lump in your throat, (11) heavy feeling in your arms or legs and (12) feeling weak in parts of your body. The selected items of the shortened version of the Profile of Mood States questionnaire are: (1) depression: unhappy, sad, hopeless, (2) vitality: active, energetic, lively, (3) anger: angry, annoyed, moody, (4) fatigue: tired, weary, fatigued and (5) tension: tense, nervous, anxious.

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